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The Major Form of ADP-Glucose Pyrophosphorylase in Maize Endosperm Is Extra-Plastidial

Kay Denyer*, Francie Dunlap, Tine Thorbjørnsen[1], Peter Keeling, and Alison M. Smith

John Innes Centre, Norwich Research Park, Colney, Norfolk NR4 7UH, United Kingdom (K.D., T.T., A.M.S.); and ExSeed Genetics LLC, 1573 Food Sciences Building, Iowa State University, Ames, Iowa 50011–1061 (F.D., P.K.)

Preparations enriched in plastids were used to investigate the location of ADP-glucose pyrophosphorylase (AGPase) in the developing endosperm of maize (*Zea mays* L.). These preparations contained more than 25% of the total activity of the plastid marker enzymes alkaline pyrophosphatase and soluble starch synthase, less than 2% of the cytosolic marker enzymes alcohol dehydrogenase and pyrophosphate, fructose 6-phosphate 1-phosphotransferase, and approximately 3% of the AGPase activity. Comparison with the marker enzyme distribution suggests that more than 95% of the activity of AGPase in maize endosperm is extra-plastidial. Two proteins were recognized by antibodies to the small subunit of AGPase from maize endosperm Brittle-2 (Bt2). The larger of the two proteins was the major small subunit in homogenates of maize endosperm, and the smaller, less abundant of the two proteins was enriched in preparations containing plastids. These results suggest that there are distinct plastidial and cytosolic forms of AGPase, which are composed of different subunits. Consistent with this was the finding that the *bt2* mutation specifically eliminated the extra-plastidial AGPase activity and the larger of the two proteins recognized by the antibody to the Bt2 subunit.

AGPase provides ADP-Glc for starch synthesis. Mutations and antisense experiments, which eliminate most or all of the activity of AGPase (Arabidopsis leaves: Lin et al., 1988; maize [*Zea mays* L.] endosperm: Tsai and Nelson, 1966; pea embryo: Hylton and Smith, 1992; potato tubers: Müller-Röber et al., 1992), lead to a severe reduction in starch content, indicating that this enzyme is the major or only route by which ADP-Glc for starch synthesis is generated. In photosynthetic cells the enzyme is considered to be located exclusively in the plastids (Okita, 1992, and refs. therein). In some nonphotosynthetic cells, such as those in soybean cell-suspension cultures (Macdonald and ap Rees, 1983), wheat endosperm (Entwistle and ap Rees, 1988), pea embryos (Smith, 1988), and oilseed rape embryos (Kang and Rawsthorne, 1994), the isolation of intact plastids has provided good evidence that AGPase is largely or exclusively plastidial. However, for maize endosperm, the situation is less clear.

There is some evidence that AGPase in maize endosperm is plastidial. First, plastids isolated from protoplasts that are derived from maize endosperm have been shown to contain AGPase activity (Echeverria et al., 1985, 1988). However, in these experiments (Echeverria, 1988) the recovery of AGPase activity was low (35%), and so they do not show whether the enzyme was confined to the plastids or whether it was present both inside and outside the plastids. Second, immunogold-labeling experiments using antibodies raised to subunits of the enzyme have led to the claim that AGPase is plastidial in maize endosperm (Miller and Chourey, 1995). There is also some evidence that the major form of AGPase may be cytosolic in the endosperms of both maize and barley. To date, the cDNAs isolated that encode the small subunit of the enzyme from maize and barley endosperm apparently lack sequences encoding transit peptides, and the proteins do not appear to undergo posttranslational cleavage (Giroux and Hannah, 1994; Villand and Kleczkowski, 1994).

To resolve the issue of AGPase location in developing barley endosperm, we recently isolated intact plastids that were largely free from cytosolic contamination (Thorbjørnsen et al., 1996). Study of the enzyme activities associated with these organelles showed that about 85% of the activity of AGPase is extra-plastidial. The use of antibodies raised to the large and small subunits of the enzyme revealed that the minor, plastidial isoform is likely to be composed of the same subunits as the isoform present in chloroplasts of barley leaves, whereas the extra-plastidial isoform is likely to be encoded by mRNAs that are abundantly and probably specifically expressed in the endosperm.

In this study we used preparations of plastids isolated from developing maize endosperm to examine the location of AGPase in the cells of this plant organ. A rapid, mechanical technique was used to maximize the chances of full recovery of enzyme activity. In conjunction with plastid preparations, we used an antibody raised to the *Bt2* gene product to locate AGPase in the endosperm of wild-type maize and in the endosperm of a *bt2* mutant that lacks the Bt2 protein.

MATERIALS AND METHODS

The wild-type line of maize (*Zea mays* L.) was the genotype UE95. The *bt2* mutant was derived from it by mu-

[1] Present address: Plant Molecular Laboratory, Agricultural University of Norway, P.O. Box 5051, N–1432 Ås, Norway.

* Corresponding author; e-mail denyerk@bbsrc.ac.uk; fax 44–1603–456844.

Abbreviations: AGPase, ADP-Glc pyrophosphorylase; Bt2, Brittle-2; DAP, days after pollination; Sh2, Shrunken-2.

tagenesis of pollen with ethyl methylsulfonate (Neuffer and Chang, 1989). It was shown to carry a mutation at the *bt2* locus by an allelism test, in which it was crossed with a known *bt2* mutant line. Plants were grown in a greenhouse at a minimum temperature of 25/20°C (day/night), supplemented with light for 15 h/d, and fertilized (21–5-20 N-P-K) once every 4 d. All plants were self-pollinated by hand, and developing ears were harvested immediately prior to the start of the experiment and kept on ice.

Plastid Preparation

All procedures were carried out at 4°C unless stated otherwise. Approximately 10 g of endosperms was removed from developing maize kernels and incubated in an extraction medium containing 0.8 M sorbitol, 50 mM Hepes (pH 7.5), 1 mM EDTA, 1 mM potassium chloride, 2 mM magnesium chloride, 2 mM DTT, and 1 g L^{-1} BSA for 20 min to 1 h. The medium was replaced with 4 to 5 mL of fresh extraction medium, and the endosperms were chopped with a razor blade. Periodically, the homogenate surrounding the chopped endosperms was removed using a wide-ended pipette and placed in a 30-mL centrifuge tube. Fresh extraction medium (4–5 mL) was added, and the endosperms were chopped further. This process of chopping, removing the homogenate, and replacing with fresh extraction medium was continued until the tissue was finely diced. The pooled homogenate (approximately 25 mL) was carefully mixed, a 1-mL sample was removed for enzyme assays, and the remainder was subjected to centrifugation at 100*g* for 10 min. The resulting pellet was resuspended in 1 mL of extraction medium. Samples of homogenate, supernatant, and pellet were mixed vigorously to rupture the plastids, centrifuged to remove starch granules and other insoluble material, and assayed immediately for enzyme activity.

Enzyme Assays

One-milliliter samples of the homogenate, supernatant, and pellet fractions were vigorously mixed to disrupt the plastids and subjected to centrifugation at 14,000*g* for 5 min. Assays were carried out on the supernatants and were performed at 25°C as follows. AGPase (EC 2.7.7.27), according to the Smith et al. (1989) assay for BC1/7RR. Alcohol dehydrogenase (EC 1.1.1.1), according to Cossins et al. (1968). The assay contained 1.3 mM NAD, 100 mM ethanol, and 85 mM glycylglycine. PPi, Fru 6-phosphate 1-phosphotransferase (EC 2.7.1.90), according to Journet and Douce (1985). Suc synthase (EC 2.4.1.13), by a modification of the method of Salerno et al. (1979). Assays contained 9 mM [U-^{14}C]UDP Glc (Amersham) at 1.85 GBq mol^{-1}, 9 mM Fru, 90 mM 3-[cyclohexylamino]-1-propanesulfonic acid (pH 9.4), and 25 μL extract in a total volume of 55 μL. Incubations (20 min at 30°C) were stopped by incubation at 100°C for 1 min. Assays, diluted to 155 μL with water, were processed using the resin method described by Jenner et al. (1994) for starch synthase assays. Alkaline pyrophosphatase (EC 3.6.1.1), according to Gross and ap Rees (1986), except that the buffer used was Bicine (pH 8.9). Soluble starch synthase (EC 2.9.1.21), according to Jenner et al. (1994) using the resin method.

SDS-PAGE and Immunoblotting

Samples were diluted with an equal volume of double-strength gel sample buffer (Laemmli, 1970) and heated to 100°C for 2 min prior to loading on gels (7.5% acrylamide, 0.75 mm thick). Gels were run in a Mini-Protean II vertical electrophoresis cell (Bio-Rad) and then blotted onto nitrocellulose according to the manufacturer's instructions. Immunoblots were probed with rabbit serum followed by alkaline phosphatase-conjugated goat anti-rabbit serum (Sigma), according to the method of Blake et al. (1984), except that the initial blocking step contained 30 g L^{-1} BSA and 20 g L^{-1} dried milk powder.

RESULTS

Most of the AGPase Activity in Developing Maize Endosperm Is Extra-Plastidial

Plastids were prepared from developing endosperm at a stage when starch had begun to accumulate (11–22 DAP). A rapid, mechanical technique similar to those used to prepare plastids from developing pea embryos (Hill and Smith, 1991), developing wheat endosperm (Tetlow et al., 1994), and developing barley endosperm (Thorbjørnsen et al., 1996) was used. The distribution of AGPase activity between the pellet and supernatant fractions was compared with the distributions of marker enzymes widely considered to be confined either to the stroma of the plastids or to the cytosol (Table I). To assess the extent of loss of activity during plastid preparation, the activity of each enzyme in the supernatant plus pellet fractions was compared with its activity in the initial homogenate. The recoveries of AGPase and most of the marker enzymes through the plastid preparation were in the range 80 to 120%, indicating that there had been no significant activation or inhibition of enzyme activity. The recoveries of soluble starch synthase activity were 120 to 130%. This high

Table I. *Activities of AGPase and marker enzymes in fractions from wild-type endosperm*

Single ears were harvested 11 to 17 DAP. Approximately 10 g of endosperm was removed from the developing kernels, and plastids were prepared as described in "Materials and Methods." All samples were assayed in duplicate. Data are the values from individual preparations or the means ± SD of values from the number of preparations shown in brackets.

Wild-Type Kernels	Activity as a Percentage of That in the Initial Homogenate	
	Pellet	Pellet + supernatant
AGPase	2.9 ± 0.6 [3]	100.5 ± 6.8 [3]
Cytosolic marker enzymes		
Alcohol dehydrogenase	1.4 ± 0.2 [3]	100.1 ± 3.6 [3]
PPi Fru 6-P 1-phosphotransferase	0.7, 0.9	82.8, 99.5
Plastidial marker enzymes		
Soluble starch synthase	47.0 ± 5.3 [3]	127.9 ± 2.2 [3]
Alkaline pyrophosphatase	24.4, 36.8	104.7, 117.6

recovery could reflect loss or dilution of inhibitory substances during plastid preparation.

The distributions of the plastidial and cytosolic marker enzymes were significantly different (Student's t test, $P < 0.001$). At least 25% of the activities of the plastid marker enzymes were recovered in the pellet, compared with less than 2% of those of the cytosolic marker enzymes. In all preparations the proportion of AGPase activity in the pellet was slightly higher than that of the cytosolic marker enzymes and considerably lower than that of the plastidial marker enzymes. These differences were statistically significant (Student's t test, $P < 0.01$), which suggests that most of the AGPase activity in maize endosperm is extra-plastidial but that a small proportion is plastidial. Based on the activity of AGPase and the percentages of the cytosolic and plastidial marker enzymes found in the pellet in three separate preparations, we estimate that 95.6 ± 1.2% (mean ± SD) of the total AGPase activity in the endosperm is extra-plastidial. This estimate is based on the assumption that the activity of AGPase in the pellet comprises both a cytosolic and a plastidial component and that these sediment to the same extent as the relevant marker enzymes. From this assumption the following equation is derived: Fraction of the total AGPase activity that is extra-plastidial = [fraction of plastidial marker enzyme in the pellet − fraction of total AGPase activity in the pellet] ÷ [fraction of plastidial marker enzyme in the pellet − fraction of cytosolic marker enzyme in the pellet].

In experiments in which more than one cytosolic or plastidial marker enzyme was assayed, the means of the values for each type of marker enzyme were used in the equation. The highest estimate of the plastidial activity of AGPase in any individual experiment was 5.5% of the total activity.

Plastidial and Extra-Plastidial AGPases May Have Small Subunits of Different Sizes

The results in Table I suggest that maize endosperm may contain two forms of AGPase, a major extra-plastidial form and a minor plastidial form. To investigate this further, we examined the distribution between the supernatant and pellet fractions of the small subunit of AGPase using an antibody to the *Bt2* gene product (the small subunit of the major AGPase of maize endosperm; a kind gift of Curtis Hannah, University of Florida, Gainesville). The antibody recognized a single band on the immunoblots of the homogenate (Fig. 1a). This protein was also present in the supernatant fraction but was not visible in the pellet fraction when the samples loaded onto the blot contained equivalent proportions (1/4300th) of a preparation. However, when the volume of the pellet fraction loaded onto the blot was increased so that the sample contained approximately the same activity of the cytosolic marker enzyme, alcohol dehydrogenase, as the sample of homogenate loaded, two proteins were recognized (Fig. 1a). In addition to the protein observed in samples of the supernatant and homogenate, the antibody also recognized a second protein of lower molecular mass. From these blots we concluded that (a) the protein recognized by the antibody in the homogenate and supernatant fractions was the Bt2 protein; (b) the Bt2 protein was largely confined to the supernatant, although a small proportion of it was also in the pellet; and (c) a second, antigenically related protein of smaller molecular mass was enriched in the pellet fraction. These results are consistent with the existence of a major, extra-plastidial form of AGPase containing the Bt2 small subunit and a minor plastidial form with a different small subunit.

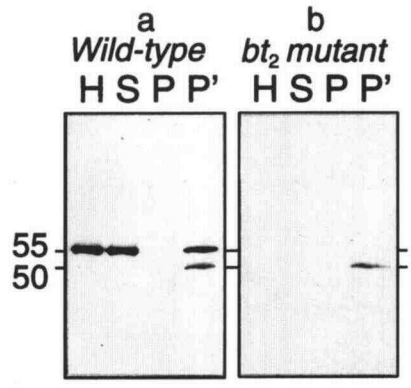

Figure 1. Immunoblots of SDS-polyacrylamide gels of fractions of wild-type (a) and *bt2* mutant (b) endosperm. Fractions were prepared as described in Tables I and II. Immunoblots were prepared as described in "Materials and Methods." Blots were probed with an antibody to the Bt2 subunit of AGPase (serum diluted by 1/15,000th). Lanes H, Homogenate; lanes S, supernatant; lanes P, pellet; and lanes P', concentrated pellet. The activities (nmol min^{-1}) of AGPase in lanes H, S, P, and P' were 0.77, 0.74, 0.019, and 1.56, respectively, for the wild-type and 0.017, not detectable, 0.004, and 0.50, respectively, for the mutant. In lanes H, S and P, the samples loaded onto the gel contained 1/4300th (wild type) or 1/5740th (*bt2* mutant) of the total volume of each fraction. For each preparation the sample loaded in lane P' contained the same activity of alcohol dehydrogenase as the sample loaded in lane H (1.34 nmol min^{-1} for the wild type and 1.00 nmol min^{-1} for the *bt2* mutant). Approximate molecular masses (determined from the positions on the blots of prestained molecular mass marker proteins) are indicated in kD on the left. Blots are typical of those obtained from several independent preparations.

The *bt2* Mutation Eliminates the Extra-Plastidial Form of AGPase

The subunits of the major AGPase in maize endosperm are encoded by genes at the *Sh2* and *Bt2* loci (Bae et al., 1990; Bhave et al., 1990). Mutations at these loci severely reduce, but do not eliminate, the AGPase activity (e.g. *bt2*: Dickinson and Preiss, 1969). To investigate the location of the residual activity, we examined the distribution of AGPase and marker enzyme activities in plastid-enriched pellets and supernatants from *bt2* mutant kernels (Table II). In these experiments the recoveries of enzyme activities in the supernatant plus pellet fractions were between 95 and 115% of those in the homogenate, suggesting that activation or inhibition of enzyme activity during plastid preparation was unlikely to influence the interpretation of the results. The AGPase activities in preparations of *bt2* kernels were considerably lower than those in preparations of wild-type kernels (Table III). In two of four preparations

Table II. *Activities of AGPase and marker enzymes in fractions from bt2 mutant endosperm*

Single ears of *bt2* mutant maize were harvested 13 to 22 DAP. Approximately 10 g of endosperm was removed, and homogenate, supernatant, and pellet fractions were prepared as described in Table I. For disrupted samples, 0.1% (v/v) Triton X-100 was included in the homogenization medium. Enzymes were assayed and data were calculated as described in Table I.

bt_2 Mutant Kernels	Activity as a Percentage of That in the Initial Homogenate			
	Pellet		Pellet + supernatant	
	Intact	Disrupted	Intact	Disrupted
AGPase	20.8, 23.9	0.3, 0.7	98.1, 71.8	103.1, 85.2
Cytosolic marker enzymes				
Alcohol dehydrogenase	1.1 ± 0.3 [4]	1.0 ± 0.3 [3]	98.7 ± 0.7 [4]	96.9 ± 8.0 [3]
Suc synthase	0.9	0.2	103.9	104.0
Plastidial marker enzymes				
Alkaline pyrophosphatase	16.1 ± 3.4 [3]	4.4 ± 7.0 [3]	102.0 ± 4.6 [3]	113.7 ± 8.6 [3]
Soluble starch synthase	35.3	0.5, 0.3	85.3	100.8, 108.3

from *bt2* kernels, the recoveries of AGPase were low. This was probably due to the difficulty of measuring the low activity in the supernatant fractions and is unlikely to reflect inactivation or inhibition of the enzyme. Table II contains only the data from the two experiments in which there was detectable AGPase activity in the supernatant fraction. However, in all four experiments, the activity of AGPase in the pellet as a percentage of the activity in the homogenate was 19 to 25%.

The distributions of marker enzymes indicated that high yields of plastids (13–35%) and low levels of cytosolic contamination (approximately 1%) were obtained in preparations from *bt2* kernels, values similar to those obtained from wild-type kernels. However, the distribution of AGPase activity was very different from that in preparations from wild-type kernels; it was not statistically significantly different from the distribution of the plastidial marker enzymes (Student's *t* test, $P > 0.1$). In preparations in which Triton X-100 (Sigma) was included in the homogenization medium to disrupt the plastids, the percentage in the pellet of the activities of both AGPase and the plastid marker enzymes was decreased considerably. The distribution of AGPase in disrupted preparations was not significantly different from the distribution of the cytosolic marker enzymes (Student's *t* test, $P > 0.1$). This suggested that, in the absence of Triton X-100, both the AGPase and the plastid marker enzymes were contained within membrane-bound organelles. Taken as a whole, these results indicated that AGPase in *bt2* mutant kernels is entirely plastidial.

The distribution of AGPase in *bt2* kernels was investigated further using the Bt2 antibody. Typical results are shown in Figure 1b. The antibody did not recognize any proteins in the homogenate, supernatant, or pellet fractions when equivalent proportions of a preparation (1/5740th) were loaded onto the gel. These samples from the *bt2* kernels contained proportions of the preparation similar to the samples of wild-type kernels shown in Figure 1a. When a more concentrated sample of the pellet fraction was loaded, a single protein of the same molecular mass as the lower of the two proteins observed in wild-type pellets was recognized (Fig. 1b, lane P'). These results indicated that, in the *bt2* line used in these experiments, the mutation eliminates the small subunit of the major, extra-plastidial form of AGPase. The presence of both the smaller protein recognized by the antibody and the activity of AGPase in plastid-enriched pellets from *bt2* mutant kernels suggested strongly that this smaller protein is the small subunit of the plastidial form of AGPase.

DISCUSSION

Our experiments show that only a small proportion of the AGPase activity in maize endosperm is plastidial. Most of the activity is extra-plastidial and is likely to be located in the cytosol. At a time in development when starch is rapidly accumulating in the endosperm and using an assay that does not include potential effectors such as phosphate or 3-phosphoglycerate, we estimate that the cytosolic activity accounts for more than 94% of the total AGPase activity.

The subunit composition of the plastidial and cytosolic forms of AGPase is different. The small subunit of the cytosolic form is the Bt2 protein. Use of an antibody raised to the product of the *Bt2* gene (Giroux and Hannah, 1994) shows that it is the major small subunit in crude homoge-

Table III. *Activities of AGPase and alcohol dehydrogenase in fractions from wild-type and bt2 mutant endosperm*

Homogenate and pellet fractions from wild-type and *bt2* mutant kernels were prepared as described in Tables I and II. Values are means ± SD of the activities of AGPase and the cytosolic marker enzyme alcohol dehydrogenase from the number of preparations shown in brackets.

Endosperm	Activity of AGPase		Activity of Alcohol Dehydrogenase	
	Homogenate	Pellet	Homogenate	Pellet
	$\mu mol\ min^{-1}$		$\mu mol\ min^{-1}$	
Wild-type kernels	8.57 ± 1.88 [3]	0.20 ± 0.08 [3]	13.2 ± 2.44 [3]	0.21 ± 0.07 [3]
bt_2 mutant kernels	0.38 ± 0.27 [4]	0.08 ± 0.07 [4]	17.5 ± 5.61 [4]	0.20 ± 0.10 [4]

nates, that it is less abundant in preparations that are enriched in plastids, and that it is eliminated by a mutation at the *Bt2* locus. The small subunit of the plastidial form is probably a protein that is recognized by the Bt2 antibody in preparations enriched in plastids. This protein is smaller than the Bt2 protein and is not affected by a mutation at the *Bt2* locus.

The subunits of the plastidial form of AGPase in maize endosperm may be encoded by *Agp1* and *Agp2*. These are genes for which cDNAs were isolated from a maize embryo library by Giroux and Hannah (1994). *Agp1* and *Agp2* (homologous to *Sh2* and *Bt2*, respectively) were found to hybridize to endosperm transcripts distinct from those of *Sh2* and *Bt2*, and the authors suggested that these or closely related genes may be responsible for the residual AGPase activity in *sh2* and *bt2* mutants. We provide evidence that the plastidial small subunit is not encoded by *Bt2* and it seems likely that it is encoded by *Agp2*. We did not investigate the location of the large subunit(s) of AGPase in maize endosperm. However, it is reasonable to suppose that the cytosolic large subunit is encoded by *Sh2* and the plastidial large subunit is encoded by *Agp1*.

Our results are not consistent with those of Miller and Chourey (1995). Based on immunolocalization only, they concluded that the major form of AGPase in developing maize endosperm is plastidial. However, their data do not unambiguously support this conclusion. First, the antibodies used gave strong immunogold labeling of the interior of starch granules in sections of developing maize endosperm. As the authors point out, AGPase is a soluble enzyme and is unlikely to be present inside starch granules. Second, two of the three antibodies used gave equally strong immunogold labeling of the cell walls. It is also unlikely that AGPase exists outside the plasma membrane. Third, the preservation of structure was not adequate to allow the stroma and cytosol to be distinguished, and the frequency of gold labeling was low throughout these regions.

Our data provide evidence that in wild-type maize endosperm both the plastidial and the cytosolic forms of AGPase contribute to the synthesis of ADP-Glc for starch synthesis. First, the activity of AGPase in the plastids is probably insufficient to account for the observed rate of starch synthesis. From published measurements (Ozbun et al., 1973; Ou-Lee and Setter, 1985) and our own unpublished data, and assuming that 5% of the AGPase activity is plastidial, we estimate that the rate of starch synthesis per kernel during the phase of rapid starch accumulation is approximately 20 to 25 nmol Glc equivalents min^{-1}, whereas the activity of AGPase in the plastids during this period is no more than 5 to 10 nmol Glc equivalents min^{-1} per kernel. Second, starch is synthesized in *bt2* mutant kernels, which, as we have shown, specifically lack activity of the cytosolic AGPase. However, the rate of starch synthesis is reduced significantly in the *bt2* mutant compared with the wild-type (Cameron and Teas, 1954).

A model for the pathway of starch synthesis in maize endosperm, based on the data we have presented, is shown in Figure 2. Essentially the same model was proposed for the pathway in developing barley endosperm by Villand and Kleczkowski (1994) and Kleczkowski (1994). ADP-Glc for starch synthesis is provided in two ways: via the cytosolic AGPase, in which case a transporter is required to transfer ADP-Glc into the plastid, and via the plastidial AGPase, in which case a supply of plastidial Glc-1-P is required. Glc-1-P may be imported directly, as in the starch-synthesizing plastids of developing wheat endosperm (Tyson and ap Rees, 1988; Tetlow et al., 1994) and potato suspension cultures (Kosegarten and Mengel, 1994), or Glc-6-P might be imported and converted to Glc-1-P by plastidial phosphoglucomutase, as in the plastids of developing pea embryos (Hill and Smith, 1991), pea roots (Borchert et al., 1989), and cauliflower buds (Neuhaus et al., 1993).

A protein that may be responsible for the import of ADP-Glc into the plastids of maize endosperm has been identified. The gene at the *Bt1* locus encodes a protein that is associated with the plastidial membranes in developing maize endosperm (Cao et al., 1995; Sullivan and Kaneko, 1995) and that shows similarity in deduced amino acid sequence to known metabolite transporters (Sullivan et al., 1991). Mutation at the *Bt1* locus results in a reduction in starch accumulation and in an increase in the ADP-Glc content of immature endosperm (Shannon et al., 1996). This increase in ADP-Glc cannot easily be explained if AGPase is assumed to be entirely plastidial. However, these effects are consistent with both the proposed role of the Bt1 pro-

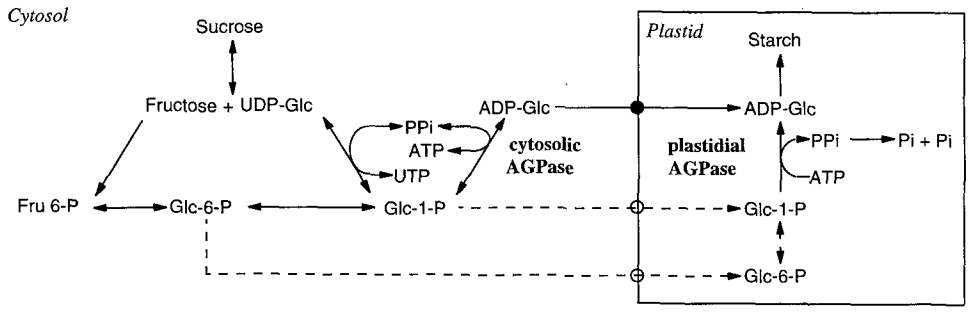

Figure 2. A model for the pathway from Suc to starch in developing maize endosperm showing the roles of the cytosolic and plastidial forms of AGPase. Two-headed arrows represent reactions that are thought to be readily reversible in vivo. Dashed arrows indicate possible routes for hexose phosphate translocation. ●, Bt1 translocator; ○; hexose phosphate translocators.

tein as an ADP-Glc transporter and the model described in Figure 2, involving AGPase activity in the cytosol.

Cytosolic and plastidial forms of AGPase also exist in barley endosperm (Thorbjørnsen et al., 1996). This raises the possibility that two forms of AGPase might exist in cereal endosperms generally. The proportion of the total AGPase activity that is plastidial is different in barley (15%) compared with maize (5%), and the proportion could be different again in the endosperm of other cereals. It may also vary through endosperm development, but this has not yet been investigated in barley or maize. The only substantial information available about the location of AGPase in any other cereal endosperm is for wheat. Data from the isolation of plastids from wheat endosperm suggest that AGPase is largely (Tetlow et al., 1994), if not entirely (Entwistle and ap Rees, 1988), plastidial. Thus, if a cytosolic AGPase exists at all in wheat endosperm, it must be a minor form. The few examples of the localization of AGPase in starch-storing organs other than cereal endosperms suggest that the enzyme in these organs also is entirely plastidial. The reason why (some) cereal endosperms possess cytosolic AGPase and other organs apparently do not remains obscure.

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